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ISOTOPE DILUTION ASSAY OF THEBAINE IN PAPAVER BRACTEATUM 2/

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1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories.

3/ This paper was submitted by Dr. E.L. Grew, Research and Development, Macfarlan Smith Ltd.

ISOTOPE DILUTION ASSAY OF THEBAINE IN P. BRACTEATUM

28.8680 g of the sample of P. bracteatum (UNB-17, as received, finely ground), 0.5357 g of C^{14}-N-methyl labelled radioactive thebaine alkaloid[4/], 150 ml of acetone, 5 g of calcium hydroxide and 15 ml of water were vigorously stirred together for 3 hours at room temperature in order to obtain uniform mixing of the active and inactive forms of the alkaloid present. The mixture was then filtered under vacuum and the filter cake washed with 100 ml of acetone. The combined filtrate and washings were evaporated to dryness in a rotary vacuum evaporator at a water-bath temperature of 40°C and the residue dissolved in chloroform (ca 100 ml). The chloroform solution was filtered to remove insoluble matter and then evaporated to ca 30 ml under reduced pressure (rotary vacuum evaporator). The resulting concentrate was stirred vigorously with 5 ml of 50% w/v tartaric acid solution for 30 minutes and the thebaine acid tartrate which crystallised out filtered off and washed several times with small portions of fresh chloroform. The crystals were dried, suspended in 10 ml of water, the mixture stirred and dilute ammonium hydroxide solution added dropwise to dissolve the solid and give a final pH of 5.0 to 6.0. One drop of a 10% aqueous solution of disodium ethylene diamine tetra-acetic acid was added followed by 0.2 g of powdered charcoal and the mixture stirred for 5 minutes and then filtered. The clear solution obtained was treated by dropwise addition of an excess of dilute ammonium hydroxide (final pH 9.0 - 9.5) with vigorous stirring in order to precipitate thebaine alkaloid. The latter was filtered off, washed well with water, and dried at 50°C. The material obtained had a melting-point of 192° - 193°C and TLC showed only a single spot with the same Rf value as thebaine, indicating that the sample was fairly pure. It can be further purified if required (and providing sufficient material is available) by recrystallisation from alcohol. The activity of the thebaine isolated was determined by planchet counting of the solid (infinitely thick layer technique) using Panax Geiger counting equipment, but liquid scintillation counting can be employed if preferred. The percentage thebaine in the original sample of P. bracteatum was calculated in the normal way (see below) and found to be 3.2%.

$$\% \text{ thebaine} = 100 \frac{W_L}{W} \left(\frac{C_L}{C_S} - 1 \right)$$

where W is the weight of the sample of P. bracteatum used

W_L is the weight of labelled thebaine used

C_L is the corrected count rate of the labelled thebaine

C_S is the corrected count rate of the sample of thebaine isolated.

4/ The labelled thebaine used had a specific activity of approximately 1.5 uC per gramme and was synthesised in the laboratories of Macfarlan Smith Ltd.